Agarose gel electrophoresis

Prepared by Ms Alex Aitken

1. Decide on the % gel needed and the number of wells and therefore the size.
2. Weigh out the Agarose needed.
3. Use a glass beaker/bottle to make up the required amount of agarose – only fill the beaker to 1/3 of its maximum volume.
4. Only make up the volume that is required for the immediate procedure.
5. DO NOT ADD A LID of any kind.
6. Place the container in the microwave on top of a piece of absorbent tissue.
7. Set the power to medium (approx 400W) NO higher.
8. Microwave in bursts of 30seconds with swirling of the mixture in between. Wear insulated gloves. Take particular care when swirling hot agarose.
9. Do not leave the microwave unattended during use.
10. Watch from a safe distance – not peering through the door. Be aware of others working in the vicinity.
11. Once the agarose has melted leave in an appropriate place to cool down clearly labelling the beaker as "hot".
12. while the agarose is cooling prepare the gel tray
    a. make sure the tray is clean
    b. tape the ends with masking tape (comes off easiest)
    c. insert the combs
13. Only pour the molten agarose once it is at a temperature comfortable to hold in your hand. If gloves are required to hold the container it is too hot to pour and should be left for longer.
14. once the gel is set (can be put at 4^{o}C for speed)
    a. remove the tape
    b. place in gel tank
    c. cover with buffer
    d. remove combs carefully
    e. Ensure buffer fills the wells.
15. If the gel remains unused for more than a day the wells should be washed with buffer (this ensures smooth loading).
16. Use an appropriately sized DNA standard.
17. Load samples using coloured-weighted dye and fluorescent DNA dye.
18. Start the electrophoresis immediately to prevent diffusion of the sample from the wells.
19. Run at an appropriate voltage for the size of the gel; too high and the gel may melt.
20. View under UV or safe light to visualise the samples.